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ANTISENSE MODULATION OF ANGIOTENSIN II $AT_1$ RECEPTOR PHYSIOLOGY IN ORGANOTYPIC CULTURES OF RAT BRAINSTEM. G.E. Gonye#, P. Hartig*, and J.S. Schwaber#. #Central Research Department, E.I. DuPont de Nemours & Co., Inc. and *Central Nervous System Diseases Research, DuPont Merck Pharmaceutical Company, Wilmington, DE 19880.

The primary afferent fibers mediating the baroreflex project onto neurons of the nucleus of the solitary tract (NTS), a nucleus containing receptors for a large variety of neuropeptides. Angiotensin II (ANG) causes well documented effects on blood pressure regulation, and $AT_1$ receptors in the medulla are expressed almost exclusively in the NTS. We have chosen the ANG/$AT_1$ system as a test system to validate a widely applicable approach to study neuropeptide-driven modulation of function at the cellular level. We have combined organotypic cultures and antisense knockdown to facilitate receptor level perturbation and subsequent physiological analysis. Knockdown of receptor expression mimics antagonists yet can circumvent the need for pharmaceutical reagents when none are available. Further, the required level of specificity demanded in receptor/ligand systems consisting of many closely related subtypes can be achieved. Organotypic brain culture has proven to be a functional intermediate between intact tissue and clonal cell lines preserving most of the organization and function of in vivo experiments. One common theme of receptor-mediated modulation, often uncovered by antisense knockdown, is a disparity of measured effects between the molecular and system levels. Small receptor binding deficits often result in much larger behavioral changes. Changes in $AT_1$ RNA and protein after culture in the presence of mismatch and antisense oligonucleotides are being determined by RT-PCR and in situ receptor autoradiography, respectively. Changes in functional coupling and cellular behavior are being determined by measuring c-fos induction and extracellularly recorded firing rate deltas after challenge with glutamate, ANG, or losartan, an $AT_1$-specific antagonist. The results of these ongoing investigations will be presented. Combining antisense knockdown with organotypic culture should prove widely applicable to ligand/receptor systems where appropriate pharmaceutical reagents are unavailable, and, ultimately, if the sole reagent available is the cDNA sequence.

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PHARMACOLOGICAL CHARACTERISTICS OF BIOACTIVE PEPTIDOMIMETIC FOR NEUROPEPTIDE Y Y2 RECEPTOR USING THE TEMPLATE ASSEMBLED SYNTHETIC PROTEINS (TASP) CONCEPT. E. Grouzmann, Centre Hospitalier Universitaire Vaudois, Division d'Hypertension, M. Mutter, Institute de Chime Organique, Université de Lausanne, Suisse.

Template assembly of potentially bioactive peptide fragments offers a convenient tool for generating molecules with agonistic/antagonistic activity. We report on a TASP molecule (TASP-Y) in which the C-terminal tetrapeptide of NPY (NPY33-36) was covalently linked via its N-terminus to a tetrafunctional template (T). Binding of this peptide has been performed on Y1 and Y2 expressing cells. The results (Table below) show that TASP-Y binds selectively to a human cell line (LN319) that expresses Y2 receptors without binding to the SK-N-MC cells that exhibit Y1 binding sites.

| Peptide | IC50 (nM) Y1 Receptor | IC50 (nM) Y2 Receptor |
|---|---|---|
| NPY1-36 | 0.5 | 0.085 |
| NPY13-36 | 1000 | 0.126 |
| NPY33-36 | >10000 | >10000 |
| TASP-Y | 4000 | 67.2 |
| T | >10000 | >10000 |

The in vitro functional activity of this molecule was investigated by measuring the intracellular free calcium concentration increase in response to NPY in LN319 cells. TASP-Y induces a shift to the right of the NPY response curve with a Ki at 43 nM. The results indicate that unlike NPY33-36, the TASP-Y analog is able to bind selectively to the Y2 receptor of NPY without any agonistic effect and is able to block the cellular response to a Y2 stimulation. Hence, TASP-Y is the first available potent and selective Y2 antagonist.

Source: https://www.industrydocuments.ucsf.edu/docs/gxcb0000